THE CONSTITUTION OF PHYSOSTIGMINE.

Thesis

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by

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The alkaloid physostigmine, or eserine, occurs in the ripe seed of Physostigma venenosum, Balfour, a leguminous plant found in West Africa. The poisonous properties of the seeds were first brought to the notice of Europeans by the publication by Daniell (Edinburgh New Philosophical Journal, 40, 319) in 1846 of the report of an expedition to the West Coast of Africa. In this report Daniell described, amongst the primitive customs of the natives of Old Calabar, a form of trial by ordeal in which the accused was compelled, usually with fatal results, either to eat the kernel itself or to drink an infusion prepared from it. Thus originated the name Ordeal Bean of Calabar, or, more briefly, Calabar Bean, by which the seed of Physostigma venenosum is now known.

Closely following the publication of Daniell's report, the botanical characters of the plant were determined by Balfour (Trans. Roy. Soc. Edinburgh, 1860, 22, 305) at Edinburgh University, whilst the physiological action of the bean was investigated, also at this University, by Fraser (Edinburgh Med. Journal, 1863, 9, 36; J. of Anat. Physiol., 1867, 1, 323; Practitioner, 1870, 4, 65). Numerous papers dealing with the physiological action either of the bean/

bean itself or of the pure alkaloid, which had meanwhile been isolated from it, were subsequently published by other authors, amongst whom the following may be mentioned: Harnack and Wilkowski (Arch. expt. Path. Pharm., 1876, 5, 401); Heubner (Arch. expt. Path. Pharm., 1905, 53, 313); Cushny (J. Physiol., 1910, 41, 244).

Physostigmine stimulates the peripheral terminations of the autonomic nerves, acting in this respect antagonistic to atropine. Its use in therapeutics is practically confined to ophthalmic practice, whereas in veterinary practice it is to some extent used as a purgative.

Despite the numerous papers which have appeared dealing with this aspect of the subject, it is, nevertheless, from Fraser's work that we derive the bulk of our knowledge of the pharmacology of the alkaloid, and it is undoubtedly to him that we owe its introduction into therapeutics.

In view of the association of Edinburgh University with the discovery and the botanical and pharmacological investigation of the Calabar bean, it would appear appropriate that attempts should also be made at this University to determine the chemical constitution of the alkaloid to which the remarkable properties of the bean are due.

The first attempts to isolate physostigmine were made by Jobst and Hesse (Annalen, 1864, 129, 115) who obtained the alkaloid in an amorphous condition. A year later Vée and Leven (J. Pharm. Chim., 1865, (IV), 1, 70) succeeded in crystallising the base, whilst Hesse (Annalen, 1867, 141, 82) in a second paper, from an analysis of the amorphous compound, assigned to it the formula $C_{15} H_{21} N_3 O_2$, a result which has been abundantly confirmed by later workers using the crystalline alkaloid.

It was shown by Petit and Polonovsky (Bull. Soc. Chim., 1893,(III.), 9, 1008) and almost simultaneously by Ehrenberg (Verh. Ges. deut. Naturforsch. Aerzte, 1893, II, 102) that physostigmine is a monacidic tertiary base, and that it also contains the grouping - CO. NH. CH_3, since, on heating with a solution of potassium hydroxide, carbon dioxide and methylamine are evolved. Ehrenberg also succeeded in isolating the residue of the alkaloid, which proved to be a new base of the composition $C_{13} H_{18} ON_2$. This was named eseroline and Ehrenberg concluded that physostigmine was a substituted carbamide which was hydrolysed by alkalis according to the following equation:-

$$(C_{13}H_{16}ON)\ NH.CO.NHCH_3 + H_2O = C_{13}H_{16}ON.NH_2 + CO_2 + NH_2CH_3$$

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It was pointed out by Salway (J.C.S., Trans., 1912, 101, 978), however, that the hydrolysis could also be explained by the presence of a urethane grouping, a view which received support from the observation of Straus (Annalen, 1913, 401, 350) that eseroline, unlike physostigmine, possessed phenolic properties. The preparation of eserethol, the ethyl ether of eseroline, by Polonovsky (Bull. Soc. Chim., 1915, (IV), 17, 235) and the partial synthesis of physostigmine by the action of methyl isocyanate upon eseroline, carried out by Polonovsky and Nitzberg (Bull. Soc. Chim., 1916,(IV), 19, 27), gave final confirmation of the urethane structure.

According to Salway (loc. cit.) eseroline is a monacidic tertiary base, and, as mentioned above, contains one hydroxyl group. In neutral and acid solution it is slowly, and in alkaline solution rapidly, oxidised by the air, different coloured products being produced according to the amount of available oxygen. Of these products, rubreserine, $C_{13}H_{16}O_2N_2$, and eserine blue, $C_{14}H_{23}O_2N_3$, have been isolated in the pure state, the former first by Ehrenberg and later by Salway, and the latter by the last-named author. The number of N-methyl groups in eseroline is uncertain. Salway, using Herzig and Meyer's/

Meyer's method, obtained results which indicated the presence of one, whilst Straus (Annalen, 1914, 406, 332), who employed Pregl's micro-method, found two such groups. An investigation of the matter by Herzig and Lieb (Monatsh., 1918, 39, 285) revealed the fact that the discrepancy between the results was due to the different proportions of hydriodic acid used. When a large excess of acid was employed, the macro-method yielded results which agreed with those of the micro-method. The suggestion was therefore made by these authors that the resistant group was possibly an ethyl group.

It is interesting to recall, in connection with this point, the experiments made by Polonovsky and Nitzberg (loc. cit.) which resulted in the partial synthesis of physostigmine. When benzene solutions of equimolecular quantities of eseroline and methyl isocyanate are heated, condensation takes place with the formation of isoeserine, a compound which is isomeric with physostigmine and still contains a free hydroxyl group. If, however, the reaction is carried out in the presence of one equivalent of sodium, condensation occurs at the hydroxyl group with the formation of physostigmine itself. Polonovsky and Nitzberg conclude that eseroline contains an imino group and that isoeserine is a substituted carbamide,

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a conclusion which receives support from further experiments made by Polonovsky (Bull. Soc. Chim., 1916, (IV), 19, 46) with phenyl isocyanate. Using this reagent he prepared, under appropriate conditions, first pheneserine, the phenyl homologue of physostigmine, and then a compound which was a condensation product of one molecule of eseroline with two of phenylisocyanate, and which could, moreover, be prepared by the action of phenyl isocyanate on pheneserine. Assuming the correctness of Polonovsky's interpretation it follows that the imino group represents the non-basic nitrogen atom, and it is difficult to conceive that this can be present in such a form except as a member of a ring. If, then, eseroline contains two N-alkyl groups these must both be attached to the tertiary basic nitrogen atom, which, consequently, cannot be cyclic. Definite evidence, however, that the basic nitrogen atom has only one methyl group attached to it exists. Thus, Polonovsky (Bull. Soc. Chim., 1917,(IV), 21, 191) found that methylamine is formed when eseroline is oxidised either by acid permanganate or dilute nitric acid. But more conclusive than this is the application, by Max and Michel Polonovsky (Bull. Soc. Chim., 1918, (IV), 23, 335), of the Hofmann degradation to eserethol methiodide. When an excess of sodium hydroxide/

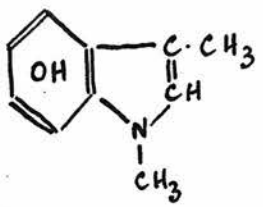
hydroxide is added to an aqueous solution of eserethol methiodide, hydriodic acid is removed with the formation of eseretholmethine, $C_{15}H_{21}N_2O$. This forms a methiodide which, when heated with concentrated potassium hydroxide, liberates trimethylamine leaving a feebly basic oil to which Polonovsky has assigned, without, apparently, an analysis, the formula $C_{14}H_{14}ON$ and the name etheseroline. These results appear quite definitely to prove that the basic nitrogen atom of eseroline forms part of a reduced ring and has but one methyl group attached to it. Consequently one is forced to conclude that there is only one N-methyl group present in eseroline.

Of the exact nature of this reduced heterocyclic ring little is known with certainty. One remarkable property has, however, been noted by Polonovsky (Bull. Soc. Chim., 1918, (IV), 23, 356). Straus (loc. cit.) had previously shown that eseroline, in the presence of finely divided platinum, absorbs two atoms of hydrogen, and concluded that this was due to the presence of a double bond. Polonovsky, using zinc and hydrochloric acid, obtained a similar result. By submitting eseroline methiodide and eserethol methine to the same treatment, the last named author found that these two compounds each absorbed two atoms of hydrogen, that the product of hydrogenation of eseroline/

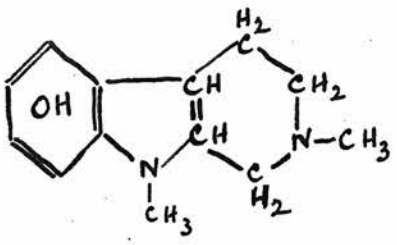
eseroline methiodide was a tertiary base in which the hydroxyl group remained intact, and further that on ethylation of this group a substance was produced which was identical with the hydrogenation product of eseretholmethine. It is clear from these results that reduction of eseroline produces a rupture of the reduced ring at the basic nitrogen atom.

It is possible that the results obtained by Straus (loc. cit.) in his attempts to apply the Hofmann degradation to eseroline methiodide may be connected with the tendency of this ring to rupture. It will be recalled that eseroline, especially in alkaline solution, readily undergoes oxidation, a property which, since it is not shared by eserethol, is obviously connected with the presence, in eseroline, of a free hydroxyl group. Straus found that the conversion of eseroline methiodide into the quaternary hydroxide intensified this effect, and it was, consequently, only by the adoption of certain artifices to prevent oxidation that he was able to carry out his experiment. Finally, by two methods, namely, distillation of methyl eserolinium hydroxide and fusion of eseroline methiodide, he was able to obtain, in poor yield, a degradation compound of the composition $C_{10}H_{11}NO$, which, on account of its phenolic properties,/

properties, he named physostigmol. When the first process was employed, a mixture of di- and trimethylamine was, at the same time, evolved, whilst only dimethylamine could be isolated from the products obtained by the second method. Physostigmol gives a red picrate and is regarded by Straus as an indole compound having the following structure:-



and on this basis he provisionally assigns to eseroline the following constitution:-



Assuming that physostigmol does actually possess the above structure, it is improbable that the same grouping exists in eseroline, for, as pointed out by Max and Michel Polonovsky (loc. cit.), physostigmol appears to be produced by a complete disintegration of eseroline rather than by simple fission; moreover, as will be shown below, the present investigation has yielded results which indicate that the hydroxyl group/

group in eseroline is attached to the ring containing the basic nitrogen atom. It appears, therefore, that an explanation of the production of physostigmol is to be sought for in the ease with which this ring undergoes rupture rather than in the existence of a benzene ring in eseroline itself. The same remarks apply to the formation of indole compounds, noted by Salway (loc. cit.), when physostigmine is distilled with zinc dust.

The presence in the Calabar bean of small quantities of other alkaloids has, from time to time, been reported (cf. Salway, J.C.S., Trans., 1911, 99, 2148). Some of these are of doubtful existence, whilst the remainder, with the exception of geneserine, which was isolated by Polonovsky and Nitzberg (Bull. Soc. Chim., 1915, (IV), 17, 244), are of little interest in connection with a discussion of the constitution of physostigmine. Geneserine, $C_{15}H_{21}N_3O_2$, is a feeble base and, on reduction, loses one atom of oxygen with the formation of physostigmine. Polonovsky and his collaborators have shown that it is an amino oxide and is, in fact, produced when hydrogen peroxide is allowed to act on physostigmine. The main interest in this new alkaloid at the present time is the further illustration which it gives of the/

the ease with which the reduced ring in physostigmine undergoes rupture. Thus, it reacts with one molecule of methyl iodide to form the hydriodide of a tertiary base, a reaction which can only be explained on the assumption that the ring is ruptured at the nitrogen atom and the oxygen atom transferred from the nitrogen to another position in the molecule.

In the present investigation a new group of degradation compounds has been obtained from eseroline. Attempts to prepare the methyl ether of eseroline methiodide by the action of sodium ethoxide and methyl iodide upon eseroline methiodide resulted in the formation, in place of the expected ether, of a methoxy-methiodide of the composition $C_{10}H_{14}N_2OI$. The name esoline is suggested for the hypothetical unmethylated tertiary base corresponding to this new methiodide, the latter thus being methyl esoline methiodide. In the formation of this compound eseroline methiodide has undergone an unexpected degradation with a nett loss of C_4H_4, and, since the hydroxyl group has been methylated in the process, at least C_5H_6 has been eliminated from the molecule. The mechanism of the reaction and the fate of the five carbon atoms are at present undetermined, but the isolation of a small quantity of what appears to be/

be an intermediate product suggests that the reaction occurs in more than one stage. No permanent gas is evolved, nor does any readily volatile substance appear to be produced. Except that it is methylated in the process, the hydroxyl group does not enter into the reaction, for, starting from eserethol methiodide, ethyl esoline methiodide has been prepared by the same process. The preparation of the last named compound also demonstrates the presence of an alkoxy group in the two compounds, further confirmation of this being provided by the removal of the alkyl group with the formation of esoline methiodide. In addition to the compounds mentioned above, methyl esoline ethiodide and ethyl esoline ethiodide have been prepared, but further investigation of this group of compounds has been confined to the two first mentioned. Methyl esoline methiodide contains two N-methyl groups, and, when converted into the quaternary hydroxide and distilled, liberates trimethylamine. It has been shown above that eseroline contains one N-methyl group; its methiodide, the starting point of the reaction under consideration, consequently contains two such groups. From the fact that this number is also present in methyl esoline methiodide, one must assume that the ring containing the basic nitrogen atom remains intact and that the non-basic nitrogen is/

is still present as an imino group. Its formula may therefore be resolved to the following:

$(NH.C_4H_4.OCH_3) > N(CH_3)_2I$.

When oxidised by nitric acid, ethyl esoline methiodide yields a quaternary nitrate of the composition $C_9H_{16}N_2O_6$, and this, by double decomposition with potassium iodide, is converted into a quaternary iodide, $C_9H_{16}NO_3I$. C_2H_3N has thus been removed, and O_2 added, to the esoline molecule during the process of oxidation. The new iodide, when converted into the quaternary hydroxide and distilled, still gives trimethylamine. From this resemblance to the esoline compounds it is probable that the reduced ring containing the basic nitrogen atom which is present in physostigmine and, apparently, also in esoline has escaped rupture during the process of oxidation. The new iodide still contains an ethoxy group, for, after heating it with hydriodic acid a small quantity of a phenolic substance has been isolated. Moreover, oxidation of methyl esoline methiodide under the same conditions gives rise to a compound which has been found, qualitatively, to differ from the present one. The form in which the additional oxygen atoms are present has not yet been determined. It was at first thought that they represented/

represented a carboxyl group, but attempts at esterification were unsuccessful. The new iodide thus contains an ethoxy group, and, presumably, two N-methyl groups. Its formula may therefore be represented thus: $C_2H_5O.\ C_5H_5O_2:\ N\ (CH_3)_2\ I$. From this formula it is clear that not more than one ring can be present in the new iodide and it follows, therefore, that the hydroxyl group in eseroline is attached to the ring containing the basic nitrogen atom. The difficulty of further interpretation of the results at the present time lies in the fact that the hydroxyl group in the free state always shows distinct phenolic properties. It must be admitted that it is difficult to reconcile this behaviour with its presence in, for example, a piperidine ring.

EXPERIMENTAL

PREPARATION OF METHYL ESOLINE METHIODIDE.

The following is an example of several similar experiments: Thirteen c.c. of a solution of 0.5 gram of sodium in 50 c.c. of ethyl alcohol were placed in a flask fitted with a reflux condenser and the air was displaced from the apparatus by means of a current of hydrogen. One gram of eseroline methiodide, prepared/

prepared by Straus's modification of Salway's method, was then added. When solution had taken place, excess of methyl iodide was added through the condenser and the flask heated on a water-bath for one and a half hours, a slow stream of hydrogen being passed through the apparatus continuously to prevent oxidation. During this period a crystalline solid separated. The solution was now cooled in ice, causing a further separation of crystals, and filtered (yield 0.3 gram). When recrystallised from methyl alcohol the compound formed prisms which commenced to darken at about 210° and melted, with decomposition, at about 235°.

Analysis.

0.1162 gram gave 0.0908 gram Ag I

0.1010 " " 0.0780 " Ag I

0.1370 " " 0.1997 " CO_2 and 0.0680 gram H_2O

* 12.344 mg. gave 0.98 cc. of N at 16° and 756 mm.

	C	H	N	I
Found	39.08	5.51	9.02	41.74) 42.19)
$C_{10}H_{14}N_2O$ I requires	38.96	5.52	9.09	41.23

* Micro-Dumas according to Dubsky.

Methyl esoline methiodide crystallises well from methyl alcohol or water. It is less soluble in ethyl/

ethyl alcohol and sparingly soluble in most other organic solvents. It gives no indole reactions, but when it is heated above its melting point under atmospheric pressure, gases and an oily distillate are formed, the former giving the pinewood reaction and the latter a very feeble indole reaction with p-dimethylaminobenzaldehyde.

ESTIMATION OF METHOXY- AND N-METHYL GROUPS IN METHYL ESOLINE METHIODIDE.

A methoxy estimation by Zeisel's method on 0.1111 gram of substance gave a negative result. The contents of the Zeisel flask were then transferred to an apparatus for the estimation of N-methyl groups by Herzig and Meyer's method. The separation of silver iodide commenced with the bath at 160°, and on a second distillation a further separation occurred at 250°. A third distillation, during which the temperature was finally raised to 300°, produced no result.

0.1111 gram gave 0.24444 gram of Ag I

Found CH_3 = 14.04%

3 CH_3 in $C_{10}H_{14}ON_2I$ requires 14.61%

The separation of silver iodide with the bath at as low a temperature as 160° suggests the presence of a methoxy group, and this is confirmed by subsequent experiments.

ATTEMPTED DEGRADATION OF METHYL ESOLINE METHIODIDE.

0.3 gram was dissolved in water and treated with an excess of moist silver oxide. Iodine was readily removed. After filtration the solution was evaporated under diminished pressure, leaving the quaternary hydroxide as a brown syrup. This was distilled under the vacuum of a water pump. Decomposition commenced at about 160°/12 mm., a volatile amine being evolved, and at 220°/12 mm. an almost colourless syrup was collected. This was dissolved in ether, but decomposed on evaporation on the water-bath. The volatile amine was collected in hydrochloric acid. It formed a hygroscopic hydrochloride and had the characteristic odour of trimethylamine, but the yield obtained was insufficient for identification. From the results of subsequent experiments it was evidently trimethylamine.

PREPARATION OF ETHYL ESOLINE METHIODIDE.

To gain information regarding the course of the reaction described above, eserethol methiodide (prepared according to Polonovsky) was submitted to the same treatment. The reaction was carried out under similar conditions, except that displacement

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of air by hydrogen was unnecessary. With a view to an improvement in yield a more concentrated solution was used. No separation of solid occurred until the experiment had been in progress for two hours; the duration was accordingly extended for a further one and a half hours, after which the solution was cooled and filtered. Using one gram of eserethol methiodide and 5 c.c. of a solution of 0.5 gram of sodium in 25 c.c. of ethyl alcohol, 0.35 gram of the crude substance was obtained. When recrystallised from methyl alcohol this formed short prisms which melted with decomposition at about 208°.

Analysis.

0.1529 gram gave 0.2311 gram of CO_2, water lost

0.2519 " " 19.0 cc. of N at 12° and 752 mm.

	C	N
Found	41.22	8.86
$C_{11}H_{19}N_2O$ I requires	41.00	8.69%

Larger quantities of this compound were subsequently required for further work. By heating the reaction mixture on the water-bath for as long as seven hours and then allowing it to stand overnight before filtering, it was found that a yield of 92% of the theoretical could be consistently obtained.

Ethyl/

Ethyl esoline methiodide is similar to methyl esoline methiodide in properties, and has a slightly greater solubility in alcohol. Unlike eseroline and eserethol it is optically inactive.

PREPARATION OF ESEROLINE ETHIODIDE.

One gram of eseroline was dissolved in ethyl iodide and allowed to stand for two days. The crystalline substance which had separated out was filtered and washed with ether (yield 1.7 gram). It was recrystallised by dissolving in the minimum amount of hot alcohol and then adding hot ethyl acetate until a faint turbidity appeared. Obtained in this manner it forms radiating needles which melt at 172°.

Analysis.

0.2397 gram gave 0.1530 gram of Ag I.

Found	I =	34.49
$C_{15}H_{23}ON_2I$	requires	33.96%

PREPARATION OF METHYL ESOLINE ETHIODIDE.

This compound was obtained from eseroline ethiodide by essentially the same method as that used for the preparation of methyl esoline methiodide.

When/

When crystallised from methyl alcohol it forms radiating aggregates which decompose at about 212°.

Analysis.

0.1483 gram gave 0.2192 gram of CO_2 and 0.0831 gram of H_2O.

	C	H
Found	40.49	6.23
$C_{11}H_{19}N_2O$ I requires	41.00	5.90

PREPARATION OF ETHYL ESOLINE ETHIODIDE.

For this purpose eserethol ethiodide was first prepared by dissolving eserethol in ethyl iodide and allowing the solution to stand until the product had crystallised out. After addition of ether, this was filtered and the crude compound so obtained used for the preparation of ethyl esoline ethiodide. The method employed was identical with that used for the preparation of ethyl esoline methiodide.

Analysis.

0.1940 gram gave 0.2982 gram of CO_2 and 0.1100 gram of H_2O.

	C	H
Found	41.92	6.30
$C_{12}H_{21}N_2O$ I requires	42.86	6.25

Ethyl esoline ethiodide forms prisms which decompose at about 201°.

EXPERIMENTS ON THE MECHANISM OF THE REACTION.

1. To determine if any permanent gases were produced, ethyl esoline methiodide was prepared in a sealed tube. The reaction proceeded normally, but no pressure was found in the tube on opening. It is therefore concluded that no permanent gases are produced.

2. Six grams of eserethol methiodide were refluxed for two hours with 24 c.c. of a solution of one gram of sodium in 50 c.c. of alcohol. No solid separated, and titration with alcoholic hydrogen chloride showed that no appreciable amount of the sodium had been used. After filtration from sodium chloride and evaporation of the alcohol the residue formed a crystalline mass. A portion of this was recrystallised and was found to be unchanged eserethol methiodide. It was subsequently used for the preparation of ethyl esoline methiodide. It is evident that, in the absence of methyl iodide, sodium ethoxide is without action on eserethol methiodide.

3. The filtrate from the preparation of ethyl esoline methiodide was fractionated. Only two fractions were obtained, which, from their boiling points, consisted of methyl iodide and ethyl alcohol respectively.

ATTEMPTED DEGREDATION OF ETHYL ESOLINE METHIODIDE.

An attempt was made to apply the Hofmann degradation to ethyl esoline methiodide using a larger quantity of material than in the corresponding experiment with methyl esoline methiodide. For this purpose, a hot aqueous solution of two grams of ethyl esoline methiodide was treated with an excess of moist, freshly precipitated, silver oxide, filtered, and the filtrate evaporated to dryness under diminished pressure. The residue formed a brown syrup having a faintly basic smell. It was transferred to a small flask and distilled under diminished pressure. The flask was fitted with a capillary which allowed a slow stream of air to pass through the apparatus and thus sweep out the whole of the volatile amine produced into hydrochloric acid contained in a vessel attached to the receiver. At about 160°/12 mm. an almost colourless oil distilled over (yield 0.55 gram).

The hydrochloric acid was evaporated to dryness on a water-bath and there was thus obtained 0.16 gram of the hydrochloride of a volatile amine. This salt was hygroscopic. After recrystallisation from alcohol it melted at 276°. A portion was then converted into the picrate, and this, after recrystallisation, melted at 218°. The remainder of the hydrochloride/

hydrochloride was converted into the platinichloride and analysed.

36.83 mg. gave 13.59 mg. of Pt.

Found Pt = 36.89

$\{(CH_3)_3 N\}_2, H_2 Pt Cl_6$ requires Pt = 36.70%

The oily distillate on treatment with aqueous picric acid gave a semi-solid picrate. This was collected and boiled with alcohol, when it became crystalline. It was recrystallised from alcohol, in which it was only sparingly soluble, and formed prisms and spherocrystals which melted at 208° - 210°.

Analysis.

0.1215 gram gave 0.2088 gram of CO_2 and 0.0461 gram of H_2O

	C	H
Found	46.87	4.22

In a second experiment six grams of ethyl esoline methiodide were converted into the quaternary hydroxide and distilled as rapidly as possible at 12 mm. Two grams of an almost colourless oil were collected. This was dissolved in ether and extracted with 1% hydrochloric acid. The ethereal solution, after drying over sodium sulphate, left on evaporation 0.2 gram of a non-basic oil. Addition of sodium hydroxide to the acid solution precipitated a basic oil/

oil which was shaken out with ether. This was dried over sodium sulphate and the solution filtered. Methyl iodide was then added when a precipitate slowly formed (yield 0.6 gram), which, when recrystallised from ethyl acetate, formed plates which melted at 172° and then solidified without melting again up to 270°.

Analysis.

0.1047 gram gave 0.0597 gram of Ag I

0.1542 " " 0.2856 " CO_2 and 0.1057 gram of H_2O

0.1738 " " 12.3 cc. of N at 11° and 735 mm.

	C	H	N	I
Found	50.51	7.62	8.15	30.82
$C_{14}H_{31}ON_2I$ requires	50.24	7.63	6.90	31.28

The ethereal mother liquors from the preparation of this iodide were evaporated to dryness and the residue heated with methyl iodide. A precipitate was formed which was filtered and recrystallised from ethyl acetate. It formed short, stout prisms which melted at 146°. Only sufficient was obtained for an iodine estimation.

Analysis.

0.1167 gram gave 0.0773 gram of Ag I.

Found I = 35.7%

In view of the similarity in the properties of these two iodides it is, perhaps, possible that complete/

complete separation was not effected. A consideration of the analyses and of the small yield of trimethylamine obtained, however, indicates that a complex change takes place. Because of this and of the small yields obtained, further material was not expended to complete and confirm the analyses.

INVESTIGATION OF THE MOTHER LIQUORS FROM THE PREPARATION OF ETHYL ESOLINE METHIODIDE.

The mother liquors obtained from the preparation of ethyl esoline methiodide were evaporated to dryness, leaving a semi-solid mass. This was dissolved in water, made alkaline with sodium hydroxide and shaken out with chloroform. After drying over sodium sulphate the chloroform was evaporated. A brown hygroscopic oil containing iodine was thus obtained which could not be induced to crystallise. It was therefore dissolved in water, shaken with freshly precipitated silver oxide, filtered, and the filtrate evaporated to dryness under diminished pressure. The residue, which was a brown oil having a strongly basic smell, was distilled in vacuo. With the bath at about 130° an obvious decomposition occurred and a small amount of a colourless liquid, which was probably/

probably water, distilled. When decomposition had ceased the temperature was raised, and at about 250°/12 mm. a yellow oil passed over, leaving a small quantity of charred material in the flask. The greater part of the oil so obtained was boiled with methyl iodide. A gelatinous solid was formed which increased in amount on the addition of ether. This was filtered and was found to be extremely soluble in alcohol and very soluble in acetone and chloroform. When precipitated from these solvents by the addition of ether it formed a gelatinous mass. It crystallised well, however, from ethyl acetate forming slender needles which softened at 132° and finally melted at 188°. In the solid state it formed a blue adsorption compound with iodine which readily dissociated in solution.

Analysis.

0.1766 gram gave 0.3030 gram of CO_2 and 0.1089 gram of H_2O

Found C = 46.79% H = 6.85%

Two drops of the distillate obtained above were dissolved in chloroform and a solution of bromine in chloroform added until it was no longer decolorised. Ether was then slowly added to the solution causing crystalline rosettes to separate. These were filtered/

filtered and recrystallised from acetone when needles melting at 203° were obtained.

The above compounds were isolated at a time when only poor yields of ethyl esoline methiodide were being obtained. When the method for the preparation of this compound was improved so that yields of over 90% were produced it was naturally found impossible to again isolate them. It is considered probable that they represent intermediate products, but unfortunately it has not so far been possible to expend further material, the whole of which was required for the preparation of ethyl esoline methiodide in the best yields obtainable, upon their further investigation.

INVESTIGATION OF THE MOTHER LIQUORS FROM THE PREPARATION OF METHYL ESOLINE METHIODIDE.

This was carried out in essentially the same manner as described above for the mother liquors from the ethyl compound. In this case a distillate was obtained with the bath at 200°. It formed a methiodide which was recrystallised from acetone and formed needles melting at 190°.

PREPARATION OF ESOLINE METHIODIDE.

0.5 gram of ethyl esoline methiodide was refluxed for half an hour with constant-boiling hydriodic acid. Oily drops of ethyl iodide condensed in the condenser and the characteristic smell of this compound was observed. The hydriodic acid was distilled off in vacuo, last traces being removed by the addition of water, which was again distilled off. The product was then dissolved in methyl alcohol and allowed to crystallise (yield 0.35 gram). After recrystallisation from water it formed long prisms which melted with decomposition at about 212°.

Analysis.

0.1541 gram gave 0.1229 gram Ag I

0.1939 " " 0.2625 gram of CO_2 and 0.0957 gram of H_2O

	C	H	I
Found	36.92	5.49	43.10
$C_9H_{15}N_2OI$ requires	36.74	5.10	43.19

Esoline methiodide possesses distinct phenolic properties. It dissolves in sodium hydroxide and is reprecipitated on acidification. With Millon's reagent it gives a faint red coloration.

OXIDATION OF ETHYL ESOLINE METHIODIDE.

In some preliminary experiments, ethyl esoline methiodide was converted into the quaternary hydroxide and oxidation of this was attempted using both potassium permanganate and nitric acid. With permanganate only unchanged material, identified by conversion into the methiodide by addition of potassium iodide, and potassium carbonate could be isolated from the reaction product. The results with nitric acid were equally unsatisfactory. From a number of experiments carried out under different conditions only minute quantities of crystalline material could be obtained. Direct oxidation of ethyl esoline methiodide with nitric acid yielded better results. After a number of preliminary experiments the following method was finally adopted.

PREPARATION OF A QUATERNARY NITRATE BY OXIDATION OF ETHYL ESOLINE METHIODIDE.

About 1 c.c. of concentrated nitric acid was added to one gram of ethyl esoline methiodide. Iodine was liberated immediately. The mixture was then gently boiled for four minutes, during which time the greater part of the iodine was driven off. Water/

Water was then added to stop the reaction and the solution again boiled until the last traces of iodine had disappeared. It was then poured on to a clock glass and evaporated to dryness on a water-bath. Crystallisation commenced spontaneously and was hastened by adding acetone and again evaporating. Obtained in this way the product forms a mass of crystals mixed with a yellow oil. It may be purified by boiling with a mixture of acetone and alcohol, which removes the yellow oil, and then recrystallising from a mixture of alcohol and ethyl acetate. It forms faintly yellow needles which melt with decomposition at about 241°.

Analysis.

0.1649 gram gave 0.2588 gram CO_2 and 0.0951 gram H_2O.

8.67 mg. " 0.855 c.c. of N at 18° and 755 m.m.

	C	H	N
Found	42.80	6.44	11.08
$C_9H_{16}N_2O_6$ requires	43.55	6.45	11.29%

The substance is very soluble in water. Its aqueous solution was treated with sodium hydroxide, evaporated to dryness, and extracted with alcohol. Addition of ether to the alcoholic extract precipitated the unchanged substance, whilst no trace of sodium nitrate could be found in the residue. This suggests that the substance is a quaternary nitrate and indicates the absence of a carboxyl group.

CONVERSION OF QUATERNARY NITRATE INTO QUATERNARY IODIDE

In conformity with the view that the preceding compound is a quaternary nitrate it was found that addition of potassium iodide to its aqueous solution caused the precipitation of a quaternary iodide. For the preparation of larger quantities of this iodide the following method was adopted. The crude nitrate obtained from one gram of ethyl esoline methiodide was dissolved in water and solid potassium iodide was added to the solution. The iodide commenced to separate almost immediately. After warming to ensure solution of the potassium iodide, the periodide which had formed was filtered and washed with water. It was then suspended in water, saturated with sulphur dioxide, and boiled until the latter had been completely driven off. This treatment decomposed the periodide and on cooling the solution the quaternary iodide crystallised out (yield 0.75 gram). It was recrystallised from methyl alcohol and again from water and formed faintly yellow, long prisms which melted at about 225° with decomposition. Even after repeated crystallisation it retained its creamy colour.

Analysis/

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Analysis.

0.1605 gram gave 0.1993 gram CO_2 and 0.0748 gram H_2O

0.2035 " " 0.2528 " " " 0.0923 " "

0.1523 " " 0.1137 " Ag I

0.3181 " " 12.6 cc. of N at 10° and 746 mm.

	C	H	N	I
Found	33.86) 33.88)	5.18) 5.04)	4.65	40.34
$C_9 H_{16} N O_3 I$ requires	34.50	5.11	4.47	40.58%

The quaternary hydroxide corresponding to this iodide formed a brown syrup which decomposed on distillation with evolution of trimethylamine, identified by its picrate, m. p. 215°. An oily distillate was also collected but was mainly basic in character. The decomposition was evidently of a complex nature, like that of the esolinium hydroxides, and was therefore not investigated further.

An attempt was made to demonstrate the presence of a carboxyl group in the iodide by esterification. 0.5 gram of the iodide was dissolved in methyl alcohol and the solution saturated with dry hydrogen chloride. After removal of the alcohol a syrup remained which was evidently a metho-chloride, for, on addition of potassium iodide a precipitate was produced. This was found to be identical with the original iodide. No esterification had, therefore, taken place.

ISOLATION OF A PHENOLIC SUBSTANCE FROM ABOVE IODIDE.

0.5 gram of the above iodide was refluxed for 30 minutes with constant boiling hydriodic acid. Drops of ethyl iodide were observed in the condenser and the characteristic smell of this substance was also detected. The hydriodic acid was then distilled off in vacuo leaving a black, sticky mass of periodide. This was dissolved in hot methyl alcohol and the solution so obtained was saturated with sulphur dioxide until almost colourless. It was then concentrated, when a small quantity of solid crystallised out on cooling. After recrystallisation from water it formed needles which melted with decomposition at about 183°. It dissolved in sodium hydroxide, and was reprecipitated from the solution on acidification. With Millon's reagent it gave a faint red coloration.

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